THE COMPLEMENT-FIXATION TEST IN TYPHOID FEVER: ITS COMPARISON WITH THE AGGLUTINATION TEST AND BLOOD CULTURE METHOD.[1]

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The diagnosis of typhoid fever has always been a subject for extensive research. In spite of the many laboratory and immunity tests at our disposal (blood culture, agglutination test, leukocyte count and differential, ophthalmo-reaction, cutaneous reaction, diazo reaction, Russo test, cultures of urine, feces, etc.), the clinician is frequently desirous of other corroborative diagnostic methods. When a positive blood culture is obtained, further examinations are unnecessary. A number of our patients, however, come to the hospital after having been ill for some time; at this stage the bacteria are not so frequently detectable in the peripheral circulation. Furthermore, under conditions like too small veins, or inadequate equipment, as is the case out of town, the blood-culture method is less practicable, and the main reliance of diagnosis must be placed upon the Widal test or the leukocyte and differential count. The former is to a certain degree losing in its importance on account of the present favorable tendency toward prophylactic typhoid inoculations, which result in a strong agglutination power of the blood. The possibility of employing the complement-fixation method as a corroborative test in the diagnosis of typhoid fever has been often suggested. The author undertook a systematic investigation of

1. The frequency of its positive finding, its time of appearance and persistence.
2. Its comparison with the agglutination test and blood culture method.
3. Its occurrence after active immunization against typhoid fever.[2]

1. Bordet and Gengou[3] (1901) were the first to demonstrate complement-fixation bodies in the serum from two convalescent typhoid patients; as antigen a saline emulsion of typhoid bacteria grown for twenty-four hours was used. Since then numerous articles referring to this topic have been published, but the conclusions reached are various.

The most favorable reports are by Widal[4] and Lesourd,[5] who out of 61 patients obtained a positive result in 58. They were unable to repeat the test in the 3 negative cases.

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[1] Read at a meeting of the New York Serological Society, October 11, 1912.

[2] This is the subject of a separate paper in preparation.

[3] Annales de l'Institut Pasteur, 1901, xv, 290.

[4] Compt.-rend. de la Soc. Biolog., 1901, liii, 841.

[5] Recherch sur la présence d'un sensibilisatrice dans le serum des typhiques, Thèse, Paris, 1902.

Lüdke[6] examined 30 patients at a time during the acute illness when the temperatures were of the high continuous type and found 7 negative; 14 cases had to be examined twice. He does not therefore attribute much importance to the diagnostic value of the test.

Posner[7] advises against its introduction because several cases gave a negative reaction, although the Widal test was positive.

Spät and Zupnik[8] report out of 18 typhoid patients, 11 positive. They found the blood culture more reliable.

Hirschfeld[9] found positive reactions in all of 15 cases. Undoubtedly this wide difference of opinion is dependent upon variation in the technique of the complement-fixation test, but to the greatest degree upon the preparation of the antigen. Here all possible modifications exist.

The oldest and most reliable antigen is the watery extract of the bacteria, advised by Wassermann and Bruck.[10] This antigen was used with excellent results in the typhoid complement-fixation studies by Hirschfeld,[11] Leuchs,[12] and Raskin.[13]

Moreschi[14] as well as Bordet and Gengou[15] used saline suspensions of typhoid bacteria.

Ficker's reagent was employed by Van Loghem;[16] Chantemesse's vaccine by Spät and Zupnik.[17] Kentzler and Kiralypi[18] prepare an ether extract of the bacteria.

The author's antigen was made according to Wassermann-Citron's[19] directions for preparing artificial aqueous aggressins. Cultures are grown for twenty-four hours upon agar slants or plates (Kolle's flasks are preferable). The growth is washed off with sterile distilled water about 1 c.c to an agar slant., The total emulsion is kept in a hot-water bath at a temperature of 60° to 65° C. for twenty-four hours, then transferred to a strong bottle and shaken vigorously with the aid of glass beads for twenty-four hours. The bacteria are thus thoroughly broken up. The mixture is next centrifugalized for a long time (four to eight hours depending upon the total quantity) until all the bacteria have sunk to the bottom and the supernatant fluid is absolutely clear. The latter is carefully pipetted off, and can be preserved for about six to eight months in sealed tubes, not exposed to sunlight or room temperature. Sterile precautions must be taken throughout the preparation. The object of using a total small quantity of distilled water is to eliminate the hemolysis of red-blood cells, which may be occasioned by employing large doses of antigen in the test.

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[6] Handbuch, Kraus and Levaditi.

[7] Berl. klin. Woch., 1908, No. 37.

[8] Berl. klin. Woch., 1908, No. 40.

[9] Zeitsch. f. klin. Med., 1907, Band lxi, Heft 3–4.

[10] Med. Klin., 1905, No. 55

[11] Loc. cit.

[12] Berl. klin. Woch., 1907, Nos. 3 and 4.

[13] Centralbl. f. Bakt., Band xlviii, Heft 4, p. 508.

[14] Berl. klin. Woch., 1906, No. 38, p. 1243.

[15] Loc. cit.

[16] Centralbl. f. Bakteriol., Band xlv, Heft 6, p. 190

[17] Loc. cit.

[18] Zeitsch. f. klin. Med., 1908, Band lxv.

[19] Immunity, Methods of Diagnosis and Therapy, Blakiston's Son Co., Philadelphia, 1913.

It is best to titrate the strength of the antigen about one week after its preparation, as after the end of this period its titer usually remains unchanged. Varying quantities of antigen are mixed with the constant units of complement, hemolysin, and red cells (sheep system), and those amounts are determined which do not of their own accord inhibit hemolysis.

TITRATION OF ANTIGEN.

Typhoid antigen. c.c.	Complement. c.c.	Hemolysin. c.c.	Red blood cells. c.c.	Saline. c.c.	Result.
0.2	0.5 (1 to 10)	0.5 (1 to 1000)	0.5 (1 to 20)	Up to 2.5	No hemolysis
0.1	0.5 (1 to 10)	0.5 (1 to 1000)	0.5 (1 to 20)	....	No hemolysis
0.05	0.5 (1 to 10)	0.5 (1 to 1000)	0.5 (1 to 20)	....	Hemolysis complete
0.025	0.5 (1 to 10)	0.5 (1 to 1000)	0.5 (1 to 20)	....	Hemolysis complete
0.0125	0.5 (1 to 10)	0.5 (1 to 1000)	0.5 (1 to 20)	....	Hemolysis complete
0.00625	0.5 (1 to 10)	0.5 (1 to 1000)	0.5 (1 to 20)	....	Hemolysis complete

In accordance with the summation law of Weil and Nakayama,[20] the dose of antigen to be employed in complement-fixation experiments is represented by one-half of the maximum quantity of antigen that does not of itself bind complement; that is, in the above instance one-half of 0.05 c.c., or 0.025 c.c., or 0.5 c.c. of a dilution 1 to 20. This unit of antigen is then tested with a known typhoid serum to prove that complement-fixation is possible.

Just as has been demonstrated by Teague and Torrey[21] and Schwartz and McNeil,[22] that for gonococcus complement-fixation work a polyvalent gonococcus antigen is necessary, and by Austrian[23] that for the typhoid ophthalmo-reaction a highly polyvalent typhoid extract is essential, so is it also of fundamental importance, in order to obtain the greatest number of positive results with the typhoid complement-fixation experiments, to have an antigen prepared from a large number of different typhoid strains. The writer is absolutely convinced of this fact. The following case proves this:

Richard, aged nine years, was admitted to the medical service of Dr. Hensel at the German Hospital in August, 1912. Probable diagnosis was typhoid fever. The blood culture disclosed bacillus typhosus, the Widal test in dilution 1 to 50 was positive in one-half hour, but the complement-fixation test was negative. It was repeated several times at intervals of a week, but remained negative. The fourth time (during the convalescence) the reaction was only doubtful. This finding seemed exceptional in comparison with the other results. An antigen was therefore prepared from the patient's own typhoid strain; the complement-fixation test performed with his own extract was strongly positive, and with the stock antigen prepared from seven different strains was doubtful; thus:

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[20] Münch. med. Woch., 1906, No. 21.

[21] Jour. Med. Res., 1907, No. 17.

[22] AMER. JOUR. MED. SCI., May, 1911.

[23] Johns Hopkins Bulletin, January, 1911.

Stock antigen.	Patient's serum.	Complement.	Hemolysin.	Red cells.	Result.
0.025 c.c.	0.1 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	±
0.05 c.c.	...	0.5 c.c.	0.5 c.c.	0.5 c.c.	−
Antigen prepared from patient's own bacteria.					
0.02 c.c.	0.1 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	++
0.01 c.c.	0.1 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	++
0.005 c.c.	0.1 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	+
0.04 c.c.	...	0.5 c.c.	0.5 c.c.	0.5 c.c.	−
...	0.2 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	−
Antigen.	Known typhoid serum.				
0.025 (stock)	0.1 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	++
0.02 (patient's)	0.1 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	++
	Known negative serum.				
0.025 (stock)	0.1 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	−
0.02 (patient's)	0.1 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	−

On reviewing the literature, the author has found that this question of specificity has been worked out experimentally by Raskin.[24] Rabbits were immunized against typhoid bacteria, each animal receiving a different strain. While the antibodies of some strains would react with an antigen made from another strain, the opposite also took place; that is, the antibodies produced by one strain would fix complement only with an extract made from that particular strain. The agglutination test was less specific.

On account of this apparent marked difference, the antigen used in the latter part of the author's (Garbat) complement-fixation work was prepared from twenty-seven different typhoid strains isolated from former patients at the hospital. (Even this number of strains could be increased.)

The *technique* of the typhoid complement-fixation reaction is the same as that in the Citron modification of the original Wassermann test, but one-half the quantities are employed:

Technique of the Reaction.

Antigen. c.c.	Serum from X. c.c.	Complement. c.c.	Hemolysin. c.c.	Sheeps' red cells. c.c.	Result.
(1) 0.025	0.1	0.5 (1 to 10)	0.5 (1 to 1000)	0.5 (1 to 20)	No hemolysis
(2) 0.0125	0.05	0.5 (1 to 10)	0.5 (1 to 1000)	0.5 (1 to 20)	No hemolysis
(3)	0.1	0.5 (1 to 10)	0.5 (1 to 1000)	0.5 (1 to 20)	Hemolysis
Controls.	Known typhoid serum.				
(1) 0.025	0.1	0.5 (1 to 10)	0.5 (1 to 1000)	0.5 (1 to 20)	No hemolysis
(2) 0.0125	0.05	0.5 (1 to 10)	0.5 (1 to 1000)	0.5 (1 to 20)	No hemolysis
(3)	0.1	0.5 (1 to 10)	0.5 (1 to 1000)	0.5 (1 to 20)	Hemolysis
	Known negative serum.				
(1) 0.025	0.1	0.5 (1 to 10)	0.5 (1 to 1000)	0.5 (1 to 20)	Hemolysis
(2) 0.0125	0.05	0.5 (1 to 10)	0.5 (1 to 1000)	0.5 (1 to 20)	Hemolysis
(3)	0.1	0.5 (1 to 10)	0.5 (1 to 1000)	0.5 (1 to 20)	Hemolysis
(4) 0.5	....	0.5 (1 to 10)	0.5 (1 to 1000)	0.5 (1 to 20)	Hemolysis

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[24] Loc. cit.

List of Cases Examined.[25]

No.	Date.	Stage of disease.	Widal test.	Blood culture.	First complement-fixation test.	Second complement-fixation test.	Third complement-fixation test.	Fourth complement-fixation test.	Remarks.
3	July 23, 1912	First to second week	+	+	4+	....	....	....	Died. Autopsy showed beginning swelling of Peyer's patches.
6	Aug. 2, 1912	" "	−	+	2+				
11	Aug. 6, 1912	" "	+	+	−	Aug. 14, −	Aug. 21, −	Aug. 28, +	
22	Sept. 14, 1912	" "	±	+	1+	Aug. 22, 3+	Sept. 2, 4+		
24	Sept. 20, 1912	" "	+	+	±	Sept. 27, +	Oct. 4, 3+		
26	Sept. 20, 1912	" "	+	+	2+	Sept. 27, 3+	Oct. 4, 4+		
4	Aug. 2, 1912	Second week	+	−	4+				
10	Aug. 10, 1912	"	±	+	−	Aug. 14, −	Aug. 21, 2+	Aug. 31, 4+	
12	Aug. 14, 1912	"	−	−	4+				
13	Aug. 14, 1912	"	−	−	−	Aug. 21, Widal, − Blood culture, − Complement-fixation test, −	Aug. 28, Widal, ± Blood culture, − Complement fixation test, 3+	Sept. 3, Widal, + Complement-fixation test, 3+	A very severe irregular typhoid. Died.
19	Aug. 28, 1912	"	+	+	3+				
21	Sept. 10, 1912	"	+	+	−	Sept. 14, −	Sept. 20, 2+		
25	Sept. 20, 1912	"	+	+	3+				
27	Sept. 20, 1912	"	+	−	−	Oct. 4, 2+			
28	Sept. 27, 1912	"	+	+	−	Oct. 4, 2+			
29	Sept. 27, 1912	"	±	+	3+				
34	Dec. 6, 1912	"	−	+	...	Dec. 13, 4+			
35	Dec. 27, 1912	"	+	+	−	Jan. 4, −	Mar. 3, 4+		
36	May 9, 1913	Third week	+	−	4+				
33	Nov. 28, 1912	"	+	+	4+				
23	Sept. 14, 1912	"	+	−	2+				
5	Aug. 2, 1912	"	+	−	4+				
9	Aug. 6, 1912	"	+	+	3+				
14	Aug. 14, 1912	"	+	−	4+				
16	Aug. 28, 1912	"	+	−	4+				
17	Aug. 28, 1912	"	+	−	3+				
18	Aug. 28, 1912	"	+	−	1+	Sept. 10, 3+			Died before repeating test. Typhoid bacteria isolated from spleen.
20	Aug. 28, 1912	"	±	−	−	....	....	....	
32	Nov. 28, 1912	Fourth week	+	−	2+				
8	Aug. 6, 1912	"	+	−	4+				
37	May 9, 1913	Eighth week	+	−	4+	....	....	....	At first diagnosed as miliary tuberculosis. Recovered.
30	Sept. 27, 1912	Convalescent	..	..	4+				
31	Sept. 27, 1912	"	+	..	4+				
1	July 23, 1912	"	..	..	4+				
2	July 23, 1912	"	..	..	4+				
7	Aug. 6, 1912	"	..	..	4+				
15	Aug. 21, 1912	"	+	−	2+				

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[25] Tabulated according to the stage of infection.

The results are reported as 4+, 3+, 2+, 1+, ±, and −, but no definite diagnosis was made on a 1+ or ± reaction. A reaction is 4+ when there is no hemolysis in tubes 1 or 2; 3+ when no hemolysis in 1, and slight hemolysis in 2; 2+ when no hemolysis in 1, and complete hemolysis in 2; 1+ when slight hemolysis in 1, and complete hemolysis in 2; ± when faint turbidity is present in 1; − when complete hemolysis exists in both 1 and 2.

In this manner 37 cases were examined in the various stages of the typhoid infection from the early onset to the late convalescent period. Out of this group, 36 sooner or later gave a strong positive reaction. If negative, the reaction was repeated every five to seven days.

25 were positive on the first examination.
5 became positive on the second examination.
5 became positive on the third examination.
1 became positive on the fourth examination.
1 remained negative. This patient died several days after admission to the hospital, so that the test was not repeated.

How Early in the Infection May the Complement-fixation Bodies Be Detected in the Blood? This answer is difficult, because of the inexactness associated with telling in what stage of the disease the patient is at a particular time. Each clinician has his own method of calculation. In the above table the author estimated this period, by counting back from the onset of abnormal temperature. That the complement-fixation test may be obtained in a very early stage of the disease was proved by Case 3, where the patient died of an extreme toxemia, and at autopsy the Peyer's patches were found just beginning to become swollen; surely not later than the end of the first or early part of the second week. In the majority of instances, however, the reaction appears toward the end of the second or early part of the third week (at a time when the blood culture tends to be negative), and becomes stronger as the disease advances toward convalescence. From the above statistics:

No of cases.	Stage of disease.	Positive results (at the first examination).
18	First and second weeks	8, or 45 per cent.
19	After second week	17, or 90 per cent.

Lüdke[26] too has found that the test appears during the second week. Hirschfeld[27] reports a positive reaction as early as the sixth to the eighth day of the infection.

How Long after the Acute Infection Does the Positive Test Persist? A greater number of cases would, of course, enable us to give a more conclusive and definite answer. As a general rule, the reaction is still obtained, and very strongly so too, six to eight weeks after the temperature has become normal. In two patients it was still positive four months afterward. One of these became a typhoid carrier with bacteria in the urine. Three patients who had typhoid fever two years previously, gave a negative reaction, although in one the Widal test was still positive (1 to 50). One patient who had an infection seven years ago and one fifteen years ago were negative. It would be of importance to test this reaction in the blood of typhoid carriers, especially those of long standing. Schöne[28] reports three such. One a carrier of ten years' standing, the second of three and one-half years, and the third of three months' duration. In all the fixation was positive. Agglutinins were present only in the third.

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[26] Loc. cit.

[27] Loc. cit.

Throughout the experiments it seemed that individuals who ran a severe or protracted type of disease developed the complement-fixation bodies less readily or later in the course than the others. While this fact holds true in a great number of instances, it cannot, however, be advocated as a guiding principle of prognosis. Thus Case 3, where the patient had a most severe infection and died several days after admission to the hospital, the complement-fixation test was strongly positive, even though, as was proved by postmortem examination, the patient was at the end of the first or beginning of the second week of the disease.

As to the clinical specificity of the reaction, sera from patients with the following diseases have been tested: La grippe, gastroduodenitis, trichiniasis, syphilis in the various stages treated and untreated, malaria, tuberculosis, pneumonia, perinephritic abscess, appendicitis, plumbism, and pelvic abscess. Numerous normal sera were also controlled. All gave a negative reaction. Occasionally a doubtful result would be noted, but never a distinct inhibition of hemolysis. One patient with an infective endocarditis, from whose blood the Streptococcus viridans was isolated, gave a positive (++) reaction on one examination, but repetition of the test with another specimen of blood taken in several days elicited a negative result. Another patient with a marked mitral endocarditis, no bacteria in the blood and only slight temperature, repeatedly gave a strong positive reaction. A history of several weeks' illness at home with high fever was obtained, but no definite diagnosis of typhoid fever. The possibility of this protracted illness having been typhoid, with the endocarditis as a sequel (there being no other distinct etiological factor) was strongly suspected. In another instance the complement-fixation test proved even more specific than the agglutination test. The serum from a case of Staphylococcus aureus sepsis with multiple abscesses of the kidney, gave an agglutination with typhoid bacteria, in dilution 1 to 50, but no complement-fixation test. (At postmortem no evidences of a typhoid infection could be discovered).

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[28] Münch. med. Woch., 1908, No. 20.

In 32 cases sufficient blood was taken at the first examination for the complement-fixation test, the Widal test, and the blood culture, so that comparative studies of the three could be made (see Table). Tabulated according to parallel results obtained, out of 18 cases during the first and second weeks of the disease and 14 cases more advanced there were:

Total number.	First and second weeks.	Later than second week.	Widal test.	Blood culture.	Complement-fixation test.
12	1	11	+	−	+
6	4	2	+	+	+
5	5	—	+	+	−
3	3	—	−	+	+
2	2	—	−	+	−
2	2	—	−	−	+
1	1	—	+	−	−
1	1	—	−	−	−

It is observed that there is no absolute uniformity in these findings. All possible combinations may occur, especially in the early stages of the disease. In the later stages, however, the general rule was a positive Widal and complement-fixation test and a negative blood culture.

If one considers the frequency with which each test by itself was positive, the following is noted:

Widal	+ in 24 cases (11 out of 18 in first two weeks, 13 out of 14 later on)
Blood culture	+ in 16 cases (14 out of 18 in first two weeks, 2 out of 14 later on)
Complement-fixation .	+ in 23 cases (10 out of 18 in first two weeks, 13 out of 14 later on)

Thus complement fixation was present as frequently as the positive Widal test, and was then of great corroborative value. In two cases (12 and 13) it was positive even before the Widal or the blood culture. Similar experiences are reported by Spät[29] and Hirschfeld.[30] That the blood culture was positive in only 50 per cent. of our cases is probably accounted for by the better class of our ward patients. They are treated at home for a longer period of time, and thus come to the hospital when there is less likelihood of still finding the bacteria in the blood.

In conclusion the following summary may be given:

1. Practically all typhoid fever patients examined, sooner or later developed antibodies in their serum, which were demonstrable by the method of complement-fixation. These bodies increase in number with the progress of the disease and are especially numerous during convalescence.

2. A highly polyvalent antigen properly prepared is absolutely essential in order to obtain a maximum of positive results.

3. Strains of typhoid bacteria may differ from each other in that their respective antibodies cannot fix complement with an antigen made from a different strain.[31]

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[29] Loc. cit.

[30] Loc. cit.

[31] With this experience in mind, the author has always employed a polyvalent typhoid vaccine for prophylactic antityphoid inoculations.

4. There is no definite relationship between the occurrence of a positive Widal and complement fixation test on one hand with a positive blood culture on the other.

5. When the blood culture is negative, as so frequently happens in the later stages of typhoid fever, a positive complement-fixation test throws great corroborative diagnostic weight on the side of a doubtful or positive Widal reaction. Occasionally the test is positive before the Widal or blood culture.

6. The very simple technique for obtaining 1 c.c. of blood from the patient's ear or finger when the veins are not suitable, stands out prominently in contrast to the extensive, and at times impossible preparations required for a blood culture. The ease of transportation of the specimen of blood must also be considered.

The writer must thank the medical attendings of the hospital for granting him the privilege of these examinations. Without their kind and constant coöperation this work would have been impossible.

RELATION OF LESIONS OF THE SMALL INTESTINE TO DISORDERS OF THE STOMACH AND CAP AS OBSERVED ROENTGENOLOGICALLY.

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The relation of lesions of the small intestine to disorders of the stomach has been recognized for many years by clinicians, most notably perhaps in the vomiting, pain, and tenderness referred to the right hypochondrium in cases of appendicitis, and in the gastric symptoms manifested by acute intestinal obstruction. Mayo has compared the stomach to a fire-alarm box, and has called attention to the futility of trying to extinguish the fire by squirting water on the alarm box. In a recent article read before the American Roentgen Ray Association,[1] Crane quoted Mayo's simile, and cited instances in which he also had observed gastro-intestinal symptoms with roentgenological evidence of a lesion at some distant point. In this communication I shall try to show that the roentgenologist can detect the first sign of smoke, and after searching farther, can usually find the location of the fire itself.

Terminology. Before entering upon any discussion an explanatory word should be said concerning terminology. While the roentgenological findings of the gastro-intestinal tract differ in every individual, I have come to recognize certain findings as

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[1] Boston, October, 1913.